



ELSEVIER

Contents lists available at ScienceDirect

## American Journal of Infection Control

journal homepage: [www.ajicjournal.org](http://www.ajicjournal.org)

AJIC  
American Journal of  
Infection Control

## Brief report

# Longitudinal characterization of *Acinetobacter baumannii-calcoaceticus* complex, *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus* colonizing and infecting combat casualties

Rebekah A. Sensenig DO<sup>a</sup>, Clinton K. Murray MD<sup>a,b</sup>, Katrin Mende PhD<sup>a,c</sup>, Steven E. Wolf MD<sup>d</sup>, Kevin K. Chung MD<sup>d</sup>, Duane R. Hospenthal MD, PhD<sup>a,b</sup>, Heather C. Yun MD<sup>a,b,\*</sup>

<sup>a</sup> Brooke Army Medical Center, Fort Sam Houston, TX

<sup>b</sup> Department of Medicine Uniformed Services University of the Health Sciences, Bethesda, MD

<sup>c</sup> Department Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences, Bethesda, MD

<sup>d</sup> United States Army Institute of Surgical Research, Fort Sam Houston, TX

## Key Words:

Colonization

Multidrug resistant

*Acinetobacter baumannii-calcoaceticus* complex*Klebsiella pneumoniae*Methicillin-resistant *Staphylococcus aureus* MRSA

Drug-resistant *Acinetobacter baumannii-calcoaceticus* complex, *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus* colonize and infect combat casualties from Iraq and Afghanistan. We retrospectively evaluated relatedness, by pulsed-field gel electrophoresis and antibiotic susceptibility testing, of isolates colonizing and infecting casualties over 2 years. Colonizing organisms were unrelated to isolates producing later infection in up to 27% of cases; most isolates underwent change in antibiotic susceptibilities. The same is true for serial infecting isolates recovered during hospitalization.

Published by Elsevier Inc. on behalf of the Association for Professionals in Infection Control and Epidemiology, Inc.

Over the last decade, a substantial increase in infections caused by multidrug-resistant organisms (MDROs) has occurred worldwide, especially among US casualties from Iraq and Afghanistan.<sup>1-3</sup> Multidrug-resistant (MDR) *Acinetobacter baumannii-calcoaceticus* complex (ABC), extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus* (MRSA) have been identified as the most commonly colonizing and infecting MDRO pathogens from combat casualty at our facility.<sup>4</sup> The high prevalence of MDRO infections in casualties from

combat operations in Iraq and Afghanistan provided a unique opportunity to study the relationship between colonizing and serial infecting isolates.

## MATERIALS AND METHODS

### Patient population and study design

Combat casualties admitted to Brooke Army Medical Center (BAMC) and the US Army Institute of Surgical Research burn center between January 2006 and September 2008 were screened (nares for MRSA, axillae and groin for gram-negative organisms) for the presence of ABC, ESBL-producing *K pneumoniae*, and MRSA.

Screening isolates were defined as organisms recovered from the nares, axillae, or groin from cultures obtained within 24 hours of admission. Infecting isolates were defined as those recovered from culture at any site during hospitalization that resulted in a clinical diagnosis of infection by the patient's physician and antibiotic treatment (excluded perioperative prophylaxis). Two separate subgroups for comparison were planned: patients with positive surveillance cultures and infecting cultures for the same organism during their hospitalization and patients with serial infecting isolates separated by  $\geq 7$  days. The electronic medical record was reviewed for presence of bacteria, type of injury, and

\* Address correspondence to Major Heather C. Yun, MD, USAF MC, Infectious Disease Service, Brooke Army Medical Center, 3851 Roger Brooke Dr, MCHE-MDI, Fort Sam Houston, TX 78234.

E-mail address: [heather.yun@amedd.army.mil](mailto:heather.yun@amedd.army.mil) (H.C. Yun).

This work has been presented in part at the 47th Annual IDSA Meeting in Philadelphia, PA, October 29-November 1, 2009.

Supported by the Global Emerging Infections Surveillance and Response System, a Division of the Armed Forces Health Surveillance Center, and by the Infectious Disease Clinical Research Program (IDCRP), of the Uniformed Services University of the Health Sciences (USUHS).

Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of the Army, Department of the Air Force, Department of Defense, or the US government. This work was prepared as part of their official duties and, as such, there is no copyright to be transferred.

Conflicts of interest: None to report.

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE <b>01 MAR 2012</b>		2. REPORT TYPE <b>N/A</b>		3. DATES COVERED <b>-</b>	
4. TITLE AND SUBTITLE <b>Longitudinal characterization of Acinetobacter baumannii-calcoaceticus complex, Klebsiella pneumoniae, and methicillin-resistant Staphylococcus aureus colonizing and infecting combat casualties</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) <b>Sensenig R. A., Murray C. K., Mende K., Wolf S. E., Chung K. K., Hospenthal D. R., Yun H. C.,</b>				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release, distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>UU</b>	18. NUMBER OF PAGES <b>3</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

**Table 1**  
Source of infecting isolates of *Acinetobacter baumannii-calcoaceticus* complex, *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus*

	Blood	Sputum	Wound	CSF	Urine	Other
ABC, n (%)						
Screening and infecting isolates (n = 33)	7 (21)	12 (36)	12 (36)	1 (3)	1 (3)	0 (0)
Serial infecting isolates (n = 134)	35 (26)	42 (31)	49 (37)	1 (1)	3 (2)	4 (3)
<i>Klebsiella pneumoniae</i> , n (%)						
Screening and infecting isolates (n = 12)	0 (0)	0 (0)	11 (99)	0 (0)	1 (1)	0 (0)
Serial infecting isolates (n = 92)	30 (33)	18 (20)	39 (42)	2 (2)	2 (2)	1 (1)
MRSA, n (%)						
Serial infecting isolates (n = 46)	30 (65)	6 (13)	9 (20)	1 (2)	0 (0)	0 (0)

ABC, *Acinetobacter baumannii-calcoaceticus* complex; CSF, cerebrospinal fluid; MRSA, methicillin-resistant *Staphylococcus aureus*.

**Table 2**  
Number of patients with genotypic and phenotypic changes of *Acinetobacter baumannii-calcoaceticus* complex and *Klebsiella pneumoniae* between initial screening and subsequent infecting isolates

	Genotypic change only	Genotypic + phenotypic change	Phenotypic change only	No change
ABC (n = 15)	1	3	4	7
Antibiotic exposure		3*	3	
<i>Klebsiella pneumoniae</i> (n = 3)	0	0	2	1
Antibiotic exposure			2	

ABC, *Acinetobacter baumannii-calcoaceticus* complex.

\*Patients with phenotypic change to an antibiotic who received that antibiotic in the interval from 7 days prior to the initial isolate through the phenotypic change were defined as having antibiotic exposure. Antibiotic received by patients who did not have phenotypic changes in their isolates between initial screening and subsequent infecting was not evaluated.

antibiotic usage. Antibiotic exposures occurring from 7 days prior to the original isolate collection through the time of recovery of subsequent isolates were considered as dichotomous variables. Statistical analysis ( $\chi^2$  testing, Fisher exact test, Kruskal-Wallis where applicable) was performed using SPSS 16.0 (SPSS, Inc, Chicago, IL). This study was approved by the BAMC Institutional Review Board.

#### Bacterial genotypic and phenotypic testing

Isolates were cultured from frozen storage ( $-80^\circ\text{C}$ ) by 2 overnight passages on blood agar (Remel, Lenexa, KS) and characterized by pulsed-field gel electrophoresis (PFGE) as previously described.<sup>5,6</sup> Genotypic change was defined as a change in 10% Dice coefficient between isolate's PFGE type. Phenotypic change was defined as a change from susceptible to intermediate or resistant or a change from intermediate or resistant to susceptible.

#### RESULTS

During the study period, 1,676 patients were admitted to BAMC from overseas combat operations, with 1,084 patients having admission screening cultures performed. Burn patients contributed 75%, 71%, and 50%, respectively, of ABC, *K pneumoniae*, and MRSA screening isolates; the remainder was predominantly orthopedic. Anatomical sources of infecting isolates are summarized in Table 1. Among all ABC and *K pneumoniae* isolates, 79% and 65% were MDR, respectively. In this cohort, 823 patients were screened for ABC specifically, and 73 (8.9%) of these were positive, of which 17 (23%) became subsequently infected, with a ratio of 4:1 colonized to infected. The evaluable screening and subsequently infecting patients/isolates, by organism, along with the changes in genotype and phenotype, are presented in Tables 2 and 3. High variability in PFGE type was demonstrated for all organisms with no apparent trends. The most frequent changes in antibiotic susceptibilities for ABC and *K pneumoniae* are summarized in Table 4. Duration of culture positivity did not correlate with either genotypic or phenotypic change of serial

ABC isolates. There was no significant correlation ( $P = .07$ ) between phenotypic change and genotypic change. Five patients had changes in antibiotic susceptibilities of the infecting MRSA isolates over time. Of these, changes from a susceptible to a resistant phenotype were seen for rifampin, fluoroquinolones, and tetracyclines.

#### DISCUSSION

Our study found that colonizing ABC isolates were unrelated to subsequent infecting isolates in over a quarter of instances, in contrast to previous reports noting that patients colonized with MDROs on admission subsequently develop infections with highly related strains.<sup>7</sup> It is also surprising that a substantial proportion of those with serial infecting isolates from the same clinical specimen source undergo a change in genotype. The finding that over half of patients demonstrated either a genotypic or phenotypic change between colonizing and subsequent infecting isolates not fully explained by antibiotic pressure suggests a large role for nosocomial transmission. However, there was no apparent trend in subsequent infecting genotypes in patients who displayed a genotypic change between colonizing and infecting isolates. This may have been evident with a larger sample size. Another possible explanation is that some patients are colonized or infected with multiple different strains of ABC at any given time, and the characterization of one colony may not be representative of the entire population.

Despite the limitation of a small number of study patients, our results showed that patients' *K pneumoniae* subsequent infecting isolates were the same strain as their original colonizing isolates, although approximately 20% did undergo genotypic change over the course of infection. However, changes in antibiotic susceptibility among isolates were normative and not explained fully by exposure to the specific antibiotic. It is likely that mechanisms effecting resistance to multiple antibiotics are partly to blame for this phenomenon, but the reversion to antibiotic susceptibility even under pressure by the antibiotic in question is

**Table 3**

Number of patients with genotypic and phenotypic changes of *Acinetobacter baumannii-calcoaceticus* complex, *Klebsiella pneumoniae*, and methicillin-resistant *Staphylococcus aureus* in serial infecting isolates

	Genotypic change only	Genotypic + phenotypic change	Phenotypic change only	No change
ABC (n = 44)	1	9	19	15
Antibiotic exposure		8*	16	
<i>Klebsiella pneumoniae</i> (n = 21)	0	4	9	8
Antibiotic exposure		3	7	
MRSA (n = 10)	0	1	4	5
Antibiotic exposure		1	2	

ABC, *Acinetobacter baumannii-calcoaceticus* complex; MRSA, methicillin-resistant *Staphylococcus aureus*.

\*Patients with phenotypic change to an antibiotic who received that antibiotic in the interval from 7 days prior to the initial isolate through the phenotypic change were defined as having antibiotic exposure. Antibiotic received by patients who did not have phenotypic changes in their isolates between initial screening and subsequent infecting was not evaluated.

**Table 4**

Antibiotic exposures and changes in susceptibilities: *Acinetobacter baumannii-calcoaceticus* complex, for amikacin, carbapenems, and tetracycline; and *Klebsiella pneumoniae*, for fluoroquinolones and piperacillin-tazobactam

<i>Acinetobacter baumannii-calcoaceticus</i> isolates changing susceptibilities during treatment						
	Amikacin		Carbapenems		Tetracycline	
	S → R	R → S	S → R	R → S	S → R	R → S
No. of isolates	3	3	2	3	3	1
No. of isolates exposed to the antibiotic of interest	3	2	2	3	3	0
<i>Klebsiella pneumoniae</i> isolates changing susceptibilities during treatment						
	Fluoroquinolones		Piperacillin-tazobactam			
	S → R	R → S	S → R	R → S		
No. of isolates	3	3	5		0	
No. of isolates exposed to the antibiotic of interest	0	1	1		N/A	

R, resistant; S, susceptible.

unusual and further supports the possibility of colonization/infection with multiple concurrent genotypes/phenotypes.

Our study has several limitations. Laboratory procedures for the storage of MDR isolates at our institution resulted in numerous isolates being unavailable for analysis, precluding inclusion in the study. A number of other factors, such as defined daily doses of antibiotics, device-days, and hospital length of stay may also have influenced strain variability and were not captured in this study. Finally, our definition of infection was clinical and not based on standardized criteria. However, this may serve to broaden applicability to the clinician considering whether to start therapy with the antimicrobial to which the patient's pathogen was susceptible last week.

Overall, these results demonstrate the ever-changing characteristics of resistant bacteria in individual patients but do not provide a clear etiology for these changes. These results demonstrate the limitations of antibiotic susceptibility patterns of a patient's prior isolate to guide subsequent empiric antibiotic therapy, given the frequent changes in antibiotic susceptibility over time.

## References

1. Scott P, Deye G, Srinivasan A, Murray C, Moran K, Hulten E, et al. An outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection in the US military health care system associated with military operations in Iraq. *Clin Infect Dis* 2007;44:1577-84.
2. Aronson NE, Sanders JW, Moran KA. In harm's way: Infections in deployed American military forces. *Clin Infect Dis* 2006;43:1045-51.
3. Bennett JW, Robertson JL, Hospenthal DR, Wolf SE, Chung KK, Mende K, et al. Impact of extended spectrum  $\beta$ -lactamase producing *Klebsiella pneumoniae* infections in severely burned patients. *J Am Coll Surg* 2010;211:391-9.
4. Murray CK, Yun HC, Griffith ME, Thompson B, Crouch HK, Monson LS, et al. Recovery of multidrug-resistant bacteria from combat personnel evacuated from Iraq and Afghanistan at a single military treatment facility. *Mil Med* 2009;174:598-604.
5. Centers for Disease Control and Prevention (CDC). PulseNet protocols. Available from: <http://www.cdc.gov/pulsenet/protocols.htm>. Accessed September 9, 2008.
6. Tenover FC, Arbeit RD, Goering RV, Mickelson PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. *J Clin Microbiol* 1995;33:2233-9.
7. Weintrob AC, Roediger MP, Barber M, Summers A, Fieberg AM, Dunn J, et al. Natural history of colonization with gram-negative multidrug-resistant organisms among hospitalized patients. *Infect Control Hosp Epidemiol* 2010;31:330-7.